

**WHAT IS CLAIMED IS:**

- 5 *Sub C1*
1. A method for fusion of expressed proteins, said method comprising the steps of:
- (a) generating at least one C-terminal thioester-tagged first target protein;
  - (b) generating at least one second target protein having a specified N-terminal; and
  - (c) ligating said first and said second target proteins.
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- 10 *Sub B1*
2. The method of claim 1, wherein said first target protein of step (a) is generated from a first plasmid comprising at least one first intein having N-terminal cleavage activity and said second target protein of step (b) is generated from a second plasmid comprising at least one second intein having C-terminal cleavage activity.
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- 15 *Sub C3*
- 20 3. The method of claim 2, wherein said first intein comprises a first modified *Mth* RIR1 intein and wherein said second modified intein comprises a second modified *Mth* RIR1 intein.
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- 25 *Sub D3*
4. The method of claim 3, wherein said first modified *Mth* RIR1 intein is selected from the group consisting of a Pro<sup>-1</sup> to Ala mutant intein, a Pro<sup>-1</sup> to Gly mutant intein, and a Pro<sup>-1</sup> - Asn<sup>134</sup> to Gly-Ala mutant intein, and wherein said second modified *Mth* RIR1 intein is selected from the group consisting of a Pro<sup>-1</sup> - Cys<sup>1</sup> to

Gly-Ser mutant intein and a Pro<sup>-1</sup> - Cys<sup>1</sup> to Gly-Ala mutant intein.

5. The method of claim 3, wherein said first plasmid is selected from the group consisting of pMRB8A, pMRB8G1 and pMRB10G, and wherein said second plasmid is selected from the group consisting of pMRB9GS, pMRB9GA and pBRL-A.

6. The method of claim 3, wherein said first target protein of step (a) is generated by thiol reagent-induced cleavage of said first modified *Mth* RIR1 intein and said second target protein of step (b) is generated by temperature and/or pH induced cleavage of said second modified *Mth* RIR1 intein.

7. The method of claim 2, wherein said specified N-terminal of step (b) comprises cysteine.

8. A method for fusion of expressed proteins, said method comprising the steps of:

- (a) constructing a first plasmid comprising at least one first target protein and at least one first modified intein, wherein said first modified intein is capable of thiol reagent-induced cleavage to produce a thioester at the C-terminal of said first target protein;

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- (b) constructing a second plasmid comprising at least one second target protein and at least one second intein having C-terminal cleavage activity, wherein said second intein is capable of cleavage to produce a said second target protein having a specified N-terminal;
- (c) generating at least one C-terminal thioester-tagged first target protein from said first plasmid of step (a);
- 10 *See B2 cont*
- (d) generating at least one second target protein having a specified N-terminal from said second plasmid of step (b); and
- (e) ligating said first target protein of step (c) with said second target protein of step (d).
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- See D5*
9. The method of claim 8, wherein step (c) further comprises purifying said C-terminal thioester-tagged first protein and step (d) further comprises purifying said second target protein having a specified N-terminal.
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10. The method of claim 9, wherein said purifications of step (c) and step (d) comprise purification on a chitin resin column.

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11. The method of claim 8, wherein said first intein of step (a) comprises a first modified *Mth* RIR1 intein, and wherein said second intein of step (b) comprises a second modified *Mth* RIR1 intein.

12. The method of claim 11, wherein said first modified *Mth* RIR1 intein is selected from the group consisting of a Pro<sup>-1</sup> to Ala mutant intein, a Pro<sup>-1</sup> to Gly mutant intein, and a Pro<sup>-1</sup> - Asn<sup>134</sup> to Gly-Ala mutant intein, and wherein said second modified *Mth* RIR1 intein is selected from the group consisting of a Pro<sup>-1</sup> - Cys<sup>1</sup> to Gly-Ser mutant intein and a Pro<sup>-1</sup> - Cys<sup>1</sup> to Gly-Ala mutant intein.

13. The method of claim 12, wherein said first plasmid of step (a) is selected from the group consisting of pMRB8A, pMRB8G1 and pMRB10G, and wherein said second plasmid of step (b) is selected from the group consisting of pMRB9GS, pMRB9GA and pBRL-A.

14. The method of claim 8, wherein said specified N-terminal comprises cysteine.

15. A fusion protein produced by the method of any one of claims 1-14.

16. A method for cyclic fusion of an expressed protein, said method comprising the steps of:  
(a) constructing a plasmid comprising at least one target protein, at least one first intein having N-terminal cleavage activity, and at least one second

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- (b) generating a C-terminal thioester-tagged target protein having a specified amino acid at its N-terminal from the plasmid of step (a); and
  - (c) intermolecular ligation of said target proteins to yield a protein polymer.
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18. The method of claim 16 or 17, wherein said first intein of step (a) comprises a first modified *Mth* RIR1 intein, and wherein said second intein of step (a) comprises a second modified *Mth* RIR1 intein.

19. The method of claim 18, wherein said first modified *Mth* RIR1 intein is selected from the group consisting of a Pro<sup>-1</sup> to Ala mutant intein, a Pro<sup>-1</sup> to Gly mutant intein, and a Pro<sup>-1</sup> - Asn<sup>134</sup> to Gly-Ala mutant intein, and wherein said second modified *Mth* RIR1 intein is selected from the group consisting of a Pro<sup>-1</sup> - Cys<sup>1</sup> to Gly-Ser mutant intein and a Pro<sup>-1</sup> - Cys<sup>1</sup> to Gly-Ala mutant intein.

20. The method of claim 16 or 17, wherein said specified amino acid comprises cysteine.

21. A cyclic protein produced by the method of any one of claim 16.

22. A modified intein comprising a mutant *Mth* R1R1 intein capable of thiol reagent-induced cleavage to produce a thioester at the C-terminal of an adjacent target protein.

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23. The modified intein of claim 22, wherein said mutant *Mth* R1R1 intein is selected from the group consisting of a Pro<sup>-1</sup> to Ala mutant intein, a Pro<sup>-1</sup> to Gly mutant intein, and a Pro<sup>-1</sup> - Asn<sup>134</sup> to Gly-Ala mutant intein.

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24. A modified intein comprising a mutant intein capable of pH and temperature-induced cleavage to produce a specified residue at the N-terminal of an adjacent target protein.

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25. The modified intein of claim 24, wherein said mutant intein comprises a mutant *Mth* R1R1 intein.

26. The modified intein of claim 25, wherein said specified residue is cysteine.

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27. The modified intein of claim 25, wherein said mutant *Mth* R1R1 intein is selected from the group consisting of a Pro<sup>-1</sup> - Cys<sup>1</sup> to Gly-Ser mutant intein and a Pro<sup>-1</sup> - Cys<sup>1</sup> to Gly-Ala mutant intein.

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28. A plasmid comprising at least one modified intein of any one of claims 22-27.

29. A plasmid comprising a modified *Mth* RIR1 intein, wherein said plasmid is selected from the group consisting of pMRB8P, pMRB8A, pMRB8G1, pMRB9GS, pMRB9GA, pMRB10G and pBRL-A.

30. A DNA segment encoding a modified *Mth* RIR1 intein, wherein said DNA segment is obtainable from a plasmid selected from the group consisting of pMRB8P, pMRB8A, pMRB8G1, pMRB9GS, pMRB9GA, pMRB10G and pBRL-A.

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